

Daniell Declaration

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Examiner : Fox, David  
Art Unit : 1638  
Applicants : Henry Daniell  
Serial No. : 09/079,640  
Docket No. : 10742-002  
Filed : May 15, 1998  
For : Universal Chloroplast Integration and Expression Vector.  
Method of Use and Transformed Plants

**DECLARATION OF HENRY DANIELL, Ph.D.**

I, Henry Daniell, Ph.D. hereby declare and say as follows:

THAT, I am employed as Pegasus Professor & Trustee Chair at University of Central Florida, Orlando, FL.;

THAT, I am the above-named Applicant and inventor of the subject matter described and claimed in the above-identified patent application;

THAT, by virtue of my educational and employment background, my leadership at national/international scientific conferences, my ongoing research, my continuing review of scientific literature, and through correspondence with professional colleagues, I am aware of the level of skill of one ordinarily skilled in the art of plant genetics, and in particular, chloroplast transformation;

THAT, I have studied the application Serial No. 09/079,640 and office actions which have been issued during prosecution of this application, as well as responses which have been filed on the Applicants' behalf, and being thus duly qualified declare as follows:

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1. The Patent Office has rejected pending claims 3, 171, 190-191, 193, 196, 214 and 216-223, as lacking enablement. I also understand that this Declaration is being filed in conjunction with a response that amends claims 190-191, *inter alia*. The statements made herein relate to the question of enablement of the aforementioned rejected claims.
2. As a foundation for discussing the rejection specified in paragraph 1, as well as other rejections, it should be helpful to clarify the terms "transcriptionally silent", "read-through" and "transcriptionally active", as used in the context of chloroplast genome spacer regions. Attention is drawn to Exhibit A, which is a diagram depicting the differences between transcriptionally active, transcriptionally silent and read-through regions in chloroplast genomes. The term "transcriptionally silent" pertains to a region located between two known divergent promoters of chloroplast genes located on opposite strands where the promoters transcribe these genes in opposite directions away from the silent region of the genome. See pages 5-6 of the application. The term "transcriptionally active" pertains to a region located between an upstream promoter and a downstream terminator. Such locations are often present in operon regions, or polycistronic transcription units, where several genes are co-transcribed by an upstream promoter. Such regions are described in the Sugita et al. (1996) reference, which is of record and has been the subject of past correspondence with the Patent Office. The term "read-through" pertains to a region that is located downstream from a terminator and upstream from a promoter. It is a known phenomenon that chloroplast terminators can be inefficient at terminating transcription. As a result, DNA introduced at a read-through region can sometimes be transcribed due to spillover of the transcript past the terminator, but such transcription is unpredictable.
3. There are a number of known transcriptionally active spacer regions in the chloroplast genome. Sixty (60) operons or polycistronic transcription units were known at the time of the filing of the present application, as was taught in the Sugita et al. (1996) reference. At the time of filing the present application, the conventional wisdom was that only regions between opposing promoters, i.e., the now defined transcriptionally

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silent regions, were appropriate for introducing foreign genes. This point has been established during the course of prosecution of the present application. However, contrary to such conventional wisdom, I persevered to determine whether one might be able to introduce and express foreign genes into regions of the chloroplast genome that were not transcriptionally silent. I discovered that the now defined "transcriptionally active" sites could successfully and reproducibly be implemented for chloroplast transformation. The results of my initial studies are provided in the present application. Since this initial work, my lab and other labs have successfully demonstrated introduction and expression of several different genetic constructs. In disproving well-accepted dogma about what regions were operable for chloroplast transformation, those skilled in the art, now equipped with the teachings provided in the present application coupled with the knowledge of those skilled in the art at the time (particularly the information provided by the Sugita et al. reference) could now test other known operon or polycistronic transcription units within the same species or genome for introduction and expression of foreign DNA. This could be achieved with straight-forward molecular biology techniques well-known at the time of filing and still in use today. Though testing "transcriptionally active" regions other than the intergenic spacer 2 region might require some experimentation, by no means would such testing of other regions be uniquely difficult or cumbersome when performed within the same species or genome. In other words, testing other transcriptionally active regions is achievable through routine experimentation and methods and would not require undue experimentation.

4. The attached Table 1 (Exhibit B) sets forth several different studies that to my knowledge demonstrate the successful chloroplast transformation utilizing the intergenic spacer 2 region, which were conducted after filing the present application. The attached Table 2 (Exhibit C) sets forth several different studies conducted after the filing of the present application that to my knowledge demonstrate successful chloroplast transformation utilizing transcriptionally silent and read-through regions. Provided in paragraph 2 is a definition of transcriptionally active, transcriptionally silent and read-through regions. Though such regions have structural differences, the same processes and mechanisms of transcription apply equally to each within the

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same species or genome. From a scientific perspective, there is nothing uniquely difficult about testing transcriptionally active regions in comparison to transcriptionally silent or read-through regions. The successful testing and implementation of numerous different transcriptionally silent and read-through regions for chloroplast transformation in fact serves as evidence that transcriptionally active spacer regions other than the intergenic spacer 2 region may also be tested and successfully implemented within the same species or genome. In my lab, there has not been a motivation to test and characterize other transcriptionally active regions because the intergenic spacer 2 regions have been successfully utilized for different chloroplast transformation studies using different constructs. However, I have no doubt that other regions could be tested and implemented with routine experimentation.

5. The undersigned declares further that all statements made herein are of his own knowledge are true and that all statements made on information in belief are believed to be true and that such willful false statements made jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.



Henry Daniell, Ph.D.



Date